A COMPARISON BETWEEN TASTE RECEPTORS AND OTHER NERVE TISSUES OF THE COCKROACH IN THEIR RESPONSES TO GUSTATORY STIMULI [1]

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It has long been known from behavioral studies that some butterflies, flies, and bees have taste receptors on the tarsi (Minnich, 1921, 1929, 1932). The electrophysiological studies reported here were begun to determine whether or not such tarsal taste receptors are present in the American cockroach, *Periplaneta americana*. In the course of these studies a clue was found to a fundamental characteristic of all taste perception in the cockroach. This is the similarity between the responses from recognized taste receptors and from nerve tissue unspecialized for taste perception. It is this relationship, briefly described earlier (Roys, 1956), which is the principal subject of this paper.

Materials

Adult male and female American cockroaches, *Periplaneta americana*, of various ages were used for all the electrophysiological experiments, while the behavioral experiments included nymphs of both sexes as well. Sex differences did not seem to have any effect on the responses in the experiments. They were all kept at room temperature and fed on powdered dog biscuit (Purina chow).

The chemicals used in the experiment were all of reagent grade except for the quinine which was U. S. P.

Nerve action potentials were picked up from the tarsal preparations through tungsten electrodes drawn to fine points in a gas-oxygen flame. The electrodes were connected by copper leads to a Grass P-3A amplifier and a Dumont 208B oscilloscope. A Grass cathode follower was also used in some of the experiments. In nerve cord preparations, bare silver electrodes were substituted for tungsten. Changes in nerve activity were measured with an Electrodyne decade impulse counter which recorded the number of nerve impulses per second. This was connected to the output of the oscilloscope amplifier.

Experiments and Results

Seven types of experiments were carried out—six on the response of various types of nerve preparations to a test substance and one on the behavioral response to the same substance presented in the drinking water. Four test substances were used—sodium chloride, hydrochloric acid, sucrose and quinine, corresponding to the four accepted taste sensations of salt, sour, sweet and bitter. First, sodium

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chloride was used in all seven types of experiments, then hydrochloric acid, sucrose and quinine, making a total of 28 different experiments. Each of these 28 was repeated at least five times to insure the validity of the results. In the following sections A through G, the rationale, techniques and results of the experiments with sodium chloride are discussed in some detail. Section H deals with substitution of hydrochloric acid, sucrose and quinine for sodium chloride in these same experiments.

A. Intact tarsus

To test whether application of sodium chloride solutions to the tarsus would produce any afferent activity in the nerve of the leg, a prothoracic leg was cut off at the femoro-tibial joint and mounted with two electrodes in contact with the nerve. One was inserted into the opening at the cut end of the leg, the other pushed in through the membrane at the tibio-tarsal joint until the tips were about one millimeter apart within the tibia. The electrodes supported the leg, and the tarsus extended down into a wax cup filled with water or test solution which could be changed with a pipette. Because of the large diameter of the electrodes relative to the size of the tibia, they usually detected afferent impulses without special care as to their exact position. The tungsten electrodes were connected to the amplifier and oscilloscope.

When the tarsus was submerged in water in the wax cup, the nerve showed a steady discharge of typical nerve spikes which probably originated in mechanoreceptors of the leg. This basal activity was measured by counting the number of spikes per second with an electronic counter set to count all spikes above the noise level. Ten consecutive counts were taken in a group and averaged. When two or more successive groups of counts showed the same level of activity, it was considered that a satisfactory base had been established. Then the water was replaced with sodium chloride in successively higher concentrations of 1, 2, 3, 4 and 5 *M*. Each concentration was left in contact with the tarsus for approximately one minute. The nerve activity continued at about the same level until a sudden increase showed that the threshold had been reached, *i.e.*, that tarsal stimulation occurred at that concentration.

Selection of the "threshold" response must, from the nature of the experiments, be somewhat arbitrary, since the basal activity showed continual small fluctuations. To be certain that the threshold was a valid one, a point was selected where the activity clearly exceeded any of the preceding base line fluctuations or any probable instrumental changes caused by shifts in line voltage, etc. Usually when a clear increase of 100% or more in the number of spikes per second occurred immediately after a change in concentration of the chemical under test and lasted longer than the quick-adapting tactile response, it was considered to be the threshold point. In some instances lower percentage increases were accepted as thresholds due to special circumstances such as an unusually even base line. Experiments were continued until five or more such thresholds at the lower end of the range coincided within half a log unit of one another. The range of the five is given in Table I and the lowest one is plotted as the threshold indicated by the bar graph in Figure 1-A.

The threshold was usually reached at 5 *M* sodium chloride. However, it was

variable and in one instance occurred at a concentration as low as 1 *M*. The roaches used in this series of experiments were all adult females, but their age as adults was unknown. If the age was the variable factor, then it seemed possible that the work of Slifer (1950) on locusts offered a further explanation of this variation in thresholds. She found that water permeability of the cuticle on locust tarsi increased with age because the impermeable outer layer was abraded away in older individuals. This suggested that permeability of the tarsal cuticle might be the

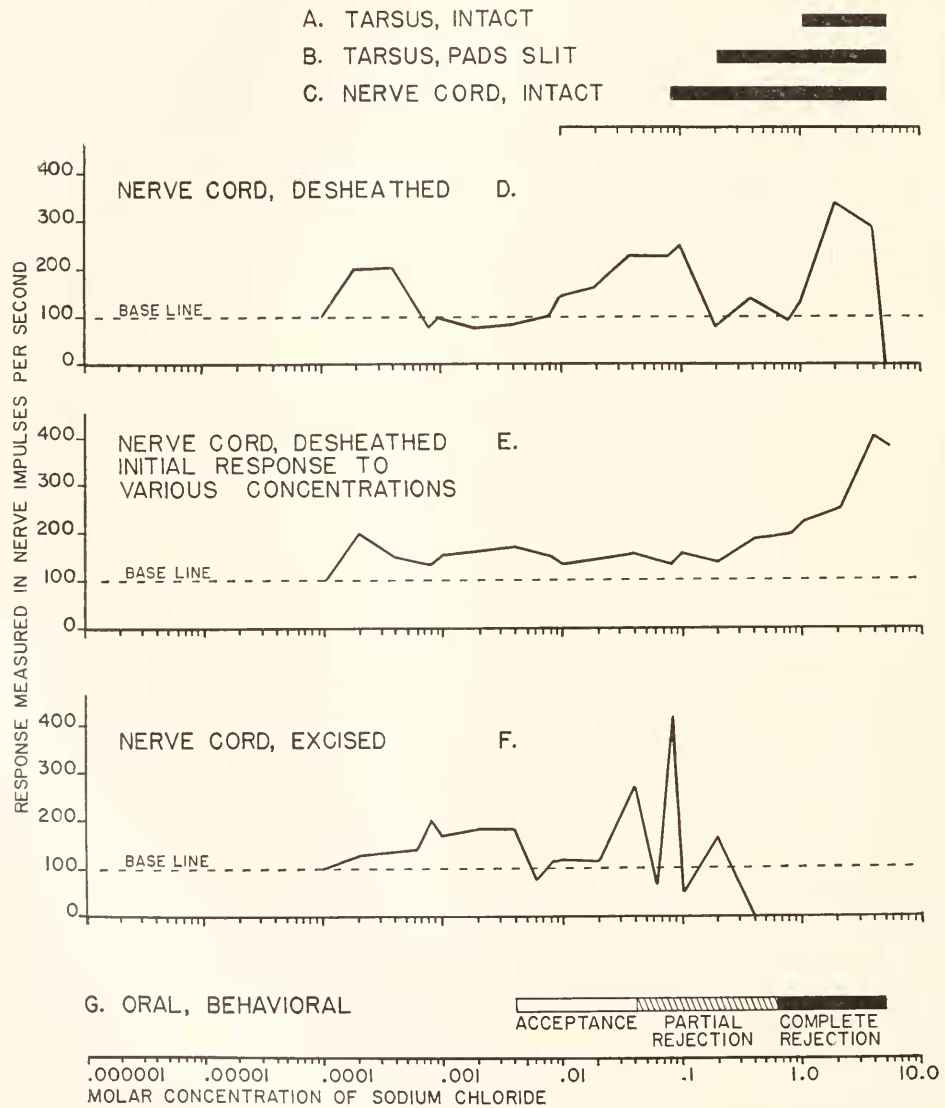

FIGURE 1. Responses of all types of preparations to sodium chloride

TABLE I

Thresholds for the four major taste qualities in different types of preparation

	Thresholds						Calculated osmotic pressure at slit tarsus thresholds
	Tarsus intact	Tarsus pads slit	Nerve cord intact	Nerve cord desheathed	Nerve cord excised	Behavioral oral	
Salt Sodium chloride	1–5 *M*	0.2–0.4 *M*	0.08–0.1 *M*	0.0002–0.0006 *M*	0.0002–0.0006 *M*	0.004–0.008 *M*	9.76 Atm.
Sour Hydrochloric acid	0.1–0.6	0.006–0.008	0.001–0.004	0.0002–0.0004	0.00001–0.00004	0.0006–0.0008	.29
Sweet Sucrose	No response at 2.5 *M*	0.1–0.4	0.1–0.6	0.002–0.004	0.001–0.004	0.006	2.44
Bitter Quinine	No response at 0.01 *M*	0.001–0.008	0.001–0.006	0.00008–0.0001	0.000006–0.000008	0.0002–0.0004	.02

factor controlling the tarsal thresholds in cockroaches. If so, slitting the tarsal pads to allow free entry of the test solution could be expected to lower the threshold.

B. Tarsus with pads slit

The tests were repeated using tarsi the five tarsal pads of which had been slit longitudinally with a razor blade. Concentrations for these experiments were increased by steps in accord with the following series: .01, .02, .04, .06, .08, .1, .2, .4, .6, .8, 1.0, etc. This progression of concentrations was also used for most subsequent experiments, but in some it was shortened to .02, .04, .08, .2, .4, .8, 2.0, etc., which approximates a doubling of concentration at each step. Because the test solutions would enter the tissue, they were made up in saline solution (9.0 g. NaCl, 0.2 g. KCl, 0.2 g. $CaCl_2$ per liter of solution; proportions from Pringle, 1938) instead of water. Thus in the sodium chloride of the test solution was included the amount of sodium chloride in saline solution plus the sodium chloride added. For example, at a threshold of 0.2 *M* sodium chloride in saline solution, the sodium chloride present was 0.15 *M* (from 9.0 g. per liter in the saline solution) plus the additional 0.2 *M*, giving a total concentration of 0.35 *M*. The same criteria for thresholds were used as for intact tarsi in the preceding series. The results are shown in Table I and Figure 1-B and it can be seen that the thresholds are much lower than for the normal intact tarsi.

C. Nerve cord in situ, intact

However, when the test solution entered the tissue of the tarsus it reached all types of nerve endings. Did the afferent response originate in special subcuticular taste receptors or in nerve endings unspecialized for taste? One way to answer

this question was to compare the responses of the tarsi to those from a nerve located where it could not normally be concerned with taste perception. Such nerves are found in the connectives and ganglia of the ventral nerve cord. If they respond to the same concentrations of sodium chloride it would indicate that the response of the tarsi was not dependent on special taste receptors.

It was found to be possible to pick up impulses from the ventral nerve cord in the intact roach simply by slipping the active electrode between the sternal plates and into the abdominal cavity just ventral to the abdominal cord. For routine experiments the roach was anesthetized with carbon dioxide, laid on its back on a small lucite block and secured in place with strips of Cenco Tackiwax. A bare tungsten electrode was inserted between the second and third abdominal sternites. The indifferent electrode was inserted in the opening formed by cutting off the tip of an antenna. Since the roach antenna contains no muscles or efferent nerves, an electrode placed there does not pick up any extraneous nerve or muscle potentials. The test solution was injected close to the cord and posterior to the pickup electrode with a fine glass pipette. Base line and threshold were established as before and the thresholds obtained are shown in Table I and Figure 1-C. They are slightly lower than those from the tarsi, indicating that the tarsal response is not entirely dependent on special taste receptors.

D. Nerve cord in situ, desheathed

Twarog and Roeder (1956) showed that the sensitivity of the roach nerve cord to chemicals was greatly increased when the connective tissue sheath was removed. To test the effect of this sheath on the sodium chloride threshold, the experiments were repeated using a modification of the Twarog window preparation. For this preparation the head and hind legs of the roach were removed, and the roach laid on its back on a plastic block and held in place by a band of Tackiwax across the thorax, leaving the ventral side of the abdomen exposed. With a fine pair of scissors two windows were cut in the ventral abdominal wall. For one, the central section of the second sternite was cut away to expose the ventral nerve cord. A loop of the exposed cord was lifted out and hung over a bare silver wire which formed the pickup electrode. As soon as the nerve cord had dried enough to stick to the wire a little, it was cut on the anterior side of the electrode to limit activity to that originating in the abdomen. When a cathode follower was used with this type of preparation it was possible to let the exposed section of nerve cord dry completely, the pickup then being through the dry dead section to the living cord inside the body (Roeder and Treat, 1957). The indifferent electrode was inserted into the body cavity at the neck. The second window was formed by cutting away the central portion of the fourth, fifth and sixth sternites to expose a nerve cord ganglion and its adjacent connectives. Under this section of cord was placed a narrow strip of Parafilm or wax paper to separate it from the underlying tissue and body fluid. The connective tissue sheath was torn with fine forceps (see Twarog and Roeder, 1956) and the exposed section perfused with saline followed by a series of test solutions. The perfusing fluid was supplied from a small reservoir through a fine glass tube and carried away by a wick of absorbent paper.

The results are shown in Table I and Figure 1-D. The base line of this and all

subsequent curves is adjusted to 100 for convenient comparison with other experiments. Curve D is taken from a single experiment considered typical of those done. The salient peaks and hollows were found in curves from all the experiments of the series, but they varied in their exact position on the x-axis so that an addition of several curves to form a composite would conceal the true form of the curve through cancellation. Hence, the single representative curve is given instead of a composite based on several experiments.

It will be noted that the thresholds are much lower than those of either intact sheathed cord or tarsus, clearly showing that the sheath checks penetration of sodium chloride and conversely that activity stimulated by these low concentrations must come from within that part of the cord normally enclosed by the sheath.

E. Nerve cord in situ, desheathed. Initial response to various concentrations

The preceding curve D, showing the response to steadily increasing concentrations of sodium chloride, has a characteristic succession of peaks and depressions. On first thought, one might interpret this to mean that the nerve does not respond to certain concentrations of sodium chloride, *e.g.*, between .0008 *M* and .008 *M*. However, it seemed more likely that these depressions represented some sort of adaptation to continued exposure to sodium chloride, and that a fresh nerve cord would respond initially to any concentration of sodium chloride above the threshold value of .0002 *M*. To check this a series of experiments was run to determine the response of a freshly dissected nerve cord in saline to each concentration shown on the curve. The results are shown in Figure 1-E. At the highest concentrations, blocking of all nerve activity began before the ten counts were completed so that the end of the curve, based on an average of ten readings, shows a drop even though the first one or two readings are higher than any preceding ones.

It can be seen from the figure that there are no concentrations above threshold to which the nerve does not initially respond, and that the extent of response generally increases with the concentration of sodium choride, particularly at high concentrations. This seems to support the inference that the depressions in curve D are due to the cumulative affect of previous treatment and do not result solely from the concentrations at which they appear.

F. Excised nerve cord

While the experiments with the normal and desheathed cord showed a clear response to rather low concentrations of sodium chloride, it may be questioned whether this response was due to direct action on the nerve cord. Possibly the perfusing fluid leaked down onto some unsuspected area of taste receptors. To check this possibility the abdominal section of the nerve cord was removed from the roach and tested alone. This consists of a chain of six ganglia joined by paired connectives and is about one centimeter long. It was dissected out and laid across silver wire electrodes in a small depression in a lucite block, in a modification of the preparation described by Roeder and Roeder (1939). When this depression was filled with saline or test solution, the cord was submerged, and no impulses were picked up because of electrical shunting through the saline solution. However, when the solution was drawn out of the depression with a small piece of absorbent

paper, the moist cord was left hanging in the air across the two electrodes and impulses were picked up for viewing on the oscilloscope and for counting.

To establish the base line activity of the cord in saline solution, it was first submerged in saline for several minutes to equilibrate with the new medium, then the solution was drawn off, ten counts of the number of spikes per second taken, and the cord submerged in saline again for one minute before another count was taken. As soon as the base level of activity had been established as for the intact tarsus, test solutions of increasing concentrations of sodium chloride were substituted for pure saline. Thus the cord was alternately submerged and exposed for periods of one minute each during the experiments, in contrast to the preceding experiments where the nerve was continually perfused. However, length of exposure to each test solution was approximately the same in both types of experiment.

Although the excised nerve cord was not desheathed, numerous openings were left wherever connectives were cut away and where the whole cord was cut at the anterior end in the process of excision. Therefore, it seems probable that the protective function of the sheath was reduced almost as much as by stripping it away.

In this preparation all six ganglia and their connectives were simultaneously exposed to each change of solution, in contrast to the preparations with the nerve cord *in situ* where only a single ganglion and its connectives were exposed. This produced responses in the excised cord which were more sharply defined and the thresholds were more easily determined than with the cord *in situ*. It also resulted in blocking at a lower concentration than that which blocked the cord *in situ*, where some of the ganglia and connectives were protected from direct exposure to the salt.

From Table I and Figure 1-F it is clear that the threshold is at least as low as that of the cord *in situ*, further confirming that no special taste receptors are needed to account for the response. Considering the preceding six types of experiment in retrospect, it is now clear that as the protective coverings are stripped away—first the cuticle to expose the leg nerve and intact cord to chemical action, then the sheath from the exposed cord—the sensitivity increases. Receptors specialized for taste are usually assumed to be more sensitive to chemicals than other nerves. However, in view of the low threshold of the desheathed nerve cord it seemed worth while to check the sensitivity of recognized taste receptors for comparison.

G. Oral taste, behavioral

Evidence from various sources and confirmed by Frings and Frings (1949) indicates that the maxillary and labial palpi of the cockroach carry the oral taste receptors. Therefore the palpi were set up in the same way as the tarsal preparations, in the expectation of getting responses in line with the behavioral thresholds reported by Frings (1946). However, the thresholds obtained were only slightly lower than those from the intact tarsi. It seems probable that this discrepancy was due to the very small size of the fibers which carry the normal gustatory responses —a condition which makes recording very difficult. To avoid this difficulty the method developed by Hodgson, Lettvin and Roeder (1955) was tried. Working

with flies they were able to make electrical contact with single sensory hairs in the oral region which responded clearly to chemical stimuli. However, this method proved unsuccessful when applied to the cockroach. The longer hairs at the tips of the palpi did not give any clear responses to gustatory stimuli, and it may be that gustatory perception is through short bristles or pegs which lie between the longer hairs and are therefore rather difficult to reach with the electrode. Therefore, for the present we must rely on the older method of behavioral response.

Behavioral thresholds for sodium chloride were determined by a modification of the method used by Dethier and Rhoades in 1954 for flies. This method offers the test population a choice between flavored and unflavored drinking water and measures the amount of each kind consumed in each of a succession of trial periods. In each trial the concentration of flavored material is greater than in the preceding one. Any change from a one-to-one ratio of consumption indicates ability to distinguish between the two, *i.e.*, the threshold concentration of the flavoring material.

In the first of these experiments a colony of about 100 adult roaches of both sexes was used for one series of experiments and a colony of nymphs for another series. However, no differences between the responses of nymphs and adults were noted and in subsequent experiments a breeding colony of mixed nymphs and adults of both sexes was used. The roaches were confined in 15-gallon aquaria containing cardboard shelters, but were not restricted in any other way and were free to eat and drink whenever they chose. Temperature ranged from 22° to 26° C. and the cages were lighted during the day by room illumination and were dark at night. This near-normal environment and lack of any restriction to movement of the experimental animal are notable advantages of this method.

The drinking water for each colony was supplied from two identical glass tubes of 6 mm. inside diameter and about 50 cm. long. These tubes lay parallel on the bottom of the aquarium except for the ends which were bent up to prevent the water from running out. At one end the bent sections came up at right angles for 12 cm. and were taped to the wall of the aquarium to hold them in position. The other two ends sloped up at 30° to a height of 1.5 cm. above the aquarium floor and were plugged with rolled cylinders of lens paper which acted as wicks to draw the water from the long horizontal reservoirs and make it available to the roaches. It was found best to put the ends of the tubes with the wicks about one centimeter apart, fastening them to a spacer block with Tackiwax to hold them firm. They were in an open area of the aquarium floor and in the light. The lens paper plugs were changed daily. Each day each tube was filled with water from a graduated syringe to a mark 1.5 cm. up on the vertical section. Thus the amount of water consumed in the preceding 24 hours was determined by measuring the amount needed to refill the tube to the mark. A colony of 100 roaches took about 10 ml. of water a day or 5 ml. from each tube if the tubes were equally preferred. Evaporation, checked in a separate tube, was nearly constant at 0.5 ml. per day. Day-to-day fluctuations in consumption from the two tubes were erratic and ran as high as 20% difference between the two with water in both tubes. To determine the salt threshold, increasing concentrations of sodium chloride (in the same steps used in the nerve preparations) were substituted for water in one of the tubes. The threshold concentration was clearly marked by a sharp increase in preference for the tube containing sodium chloride, *i.e.*, an acceptance threshold.

This was followed by a continued preference for the sodium chloride tube at higher concentrations until the rejection threshold was reached when there was a sharp change in preference from sodium chloride to water. Higher concentrations of salt were progressively less and less acceptable until a concentration was reached at which no salt solution at all was taken, *i.e.*, salt was completely rejected at or above that concentration. Supplementary experiments showed that previous conditioning had little effect on any given trial. For example, in a given pair of tubes when an unacceptable sodium chloride solution was replaced by water, there was an immediate return to an approximately one-to-one ratio of preference and the same appeared to be true in shifting from an acceptable solution to water.

Two complete series, ranging from well below the acceptance threshold to well above the point of complete rejection, were run. In addition three short series were run in the ranges of the acceptance and rejection thresholds. The results are shown in Table I and the bar graph in Figure 1-G, and it is clear from these that the behavioral threshold is well above that of any of the nerve cord preparations.

H. Responses from all types of preparation to sour, sweet and bitter stimuli

To test whether comparable thresholds and curves could also be obtained from sour, sweet and bitter substances, similar series of tests were carried out with hydrochloric acid, sucrose and quinine. Quinine monohydrochloride was substituted for quinine at concentrations above 0.001 *M* because of its greater solubility. At lower concentration it had the same threshold as the pure alkaloid. The experiments with sucrose and quinine were not carried out to 5.0 *M* because sucrose solution becomes a thick syrup above 2.0 *M* concentration, while quinine monohydrochloride tends to precipitate out of solution at concentrations much above 0.01 *M* in water. In every other way the same procedure was followed as for sodium chloride.

The results of these experiments are shown in Table I and Figures 2, 3 and 4. Figure 5 is a summary of all the thresholds for all four substances, plotted on a single graph for comparison.

In comparing the responses to hydrochloric acid with those to sodium chloride it is at once apparent that most of the hydrochloric acid thresholds are lower. An apparent exception is found in the thresholds of the desheathed nerve cord *in situ*. This discrepancy is probably due to a limitation of technique rather than a real physiological difference. This technical limitation lies in the fact that much less area is exposed to test solutions with the cord *in situ* than with it excised (see section F). For this reason the responses from the cord *in situ* were less clearly defined and the threshold less easily determined. In the cases of sodium chloride and sucrose the experimental evidence seemed to support thresholds of the cord *in situ* as low as those of the excised cord. With hydrochloric acid and quinine the evidence at hand did not seem to warrant a firm statement to this effect, although there is a strong probability that it is true for these substances also.

As with sodium chloride, the behavioral tests with hydrochloric acid showed both acceptance and rejection thresholds, and concentrations causing rejection also stimulated the tarsi and produced a strong response in the nerve cord.

Sucrose did not stimulate the intact tarsus in concentrations up to 2.0 *M*, and

thresholds for all types of preparation were rather high. In the behavioral tests sucrose was acceptable at all concentrations from threshold to 2.0 *M*.

Quinine also failed to stimulate the intact tarsi, suggesting that the cuticle is less permeable to the large molecules of sucrose and quinine than to the ionized salts and acids. In behavioral tests quinine was rejected in all concentrations above the threshold.

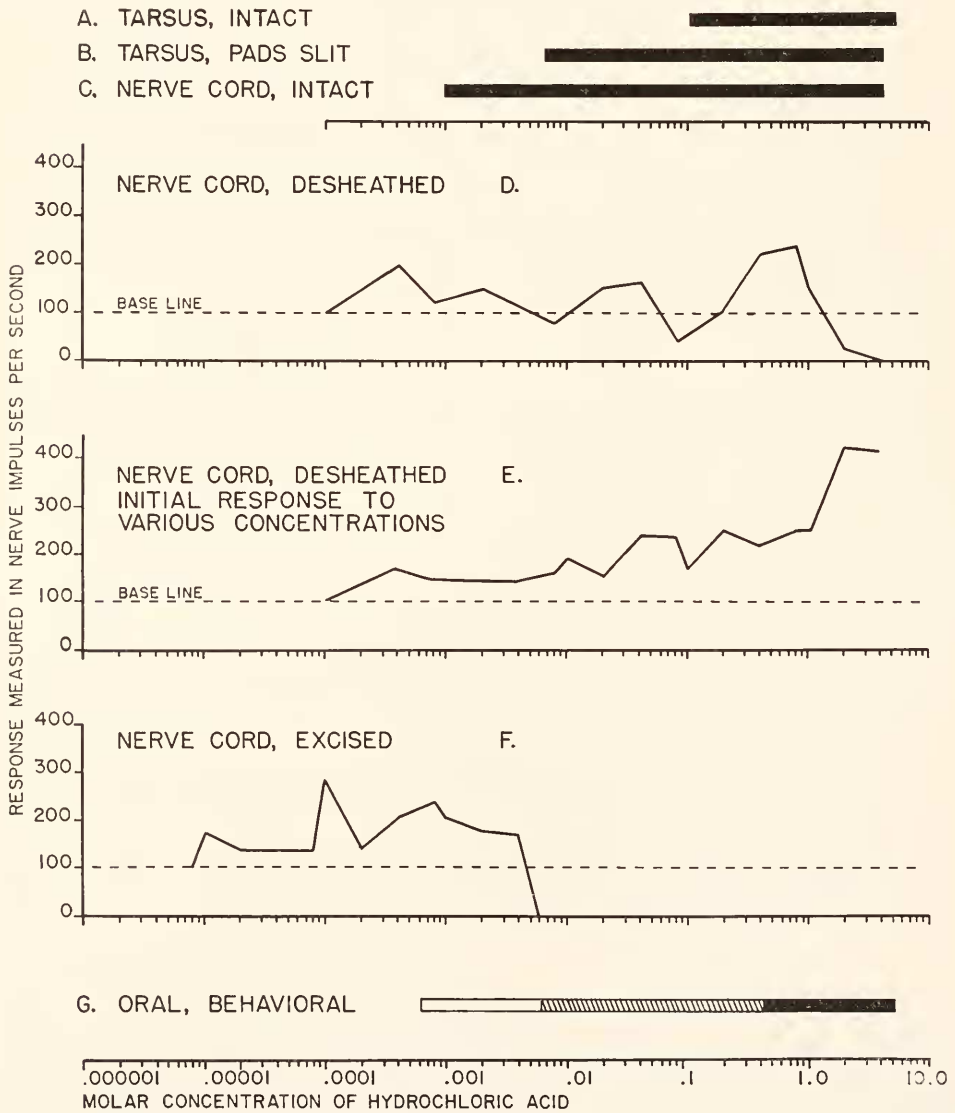

FIGURE 2. Responses of all types of preparations to hydrochloric acid. Legend for bar: unshaded area, acceptance; cross-hatched area, partial rejection; black area, complete rejection.

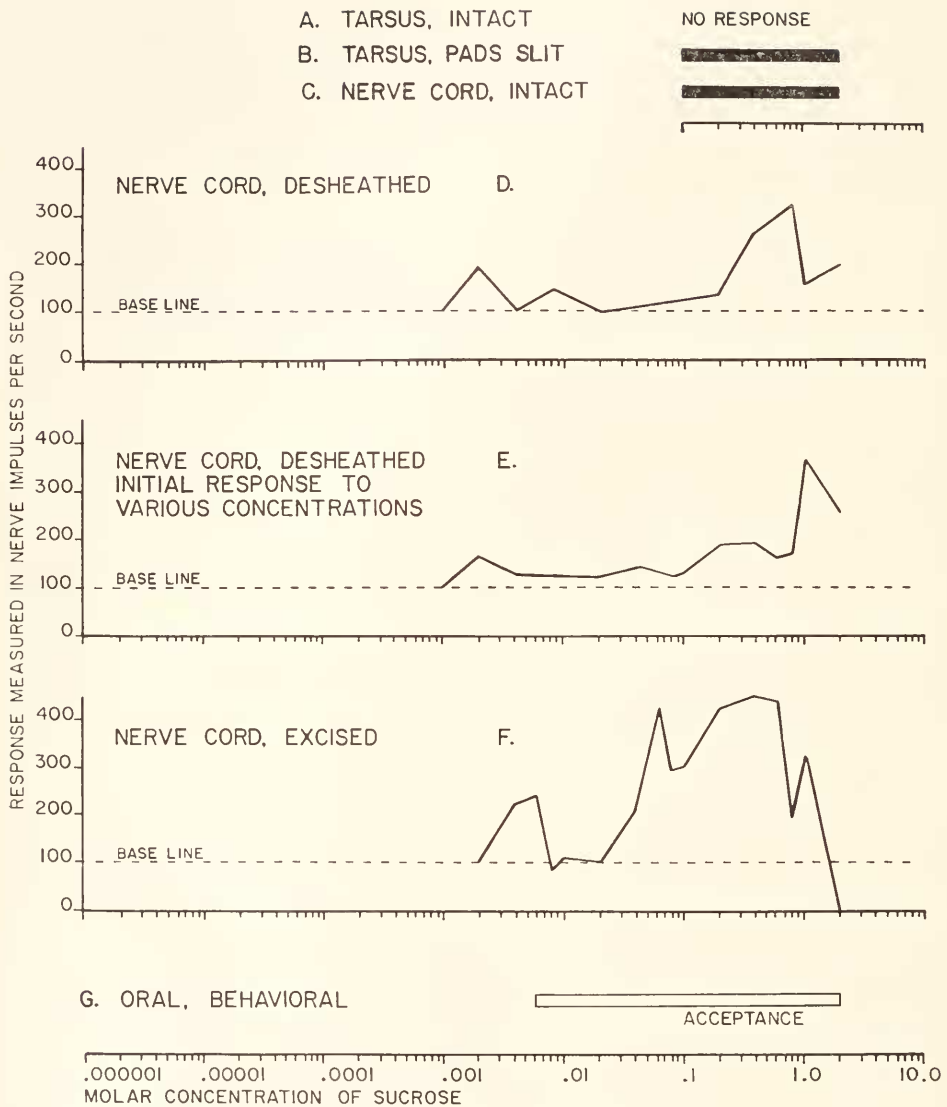

FIGURE 3. Responses of all types of preparations to sucrose.

DISCUSSION

The foregoing sets of experiments seem to indicate that the chemical sensitivity of recognized oral taste receptors, as well as receptors on the tarsi, is always less than that of many other nerves in the body not normally concerned with taste. However, there are a number of points in the work which may need clarification or can profitably be amplified.

The first consideration is whether the nerve responses obtained are true chem-

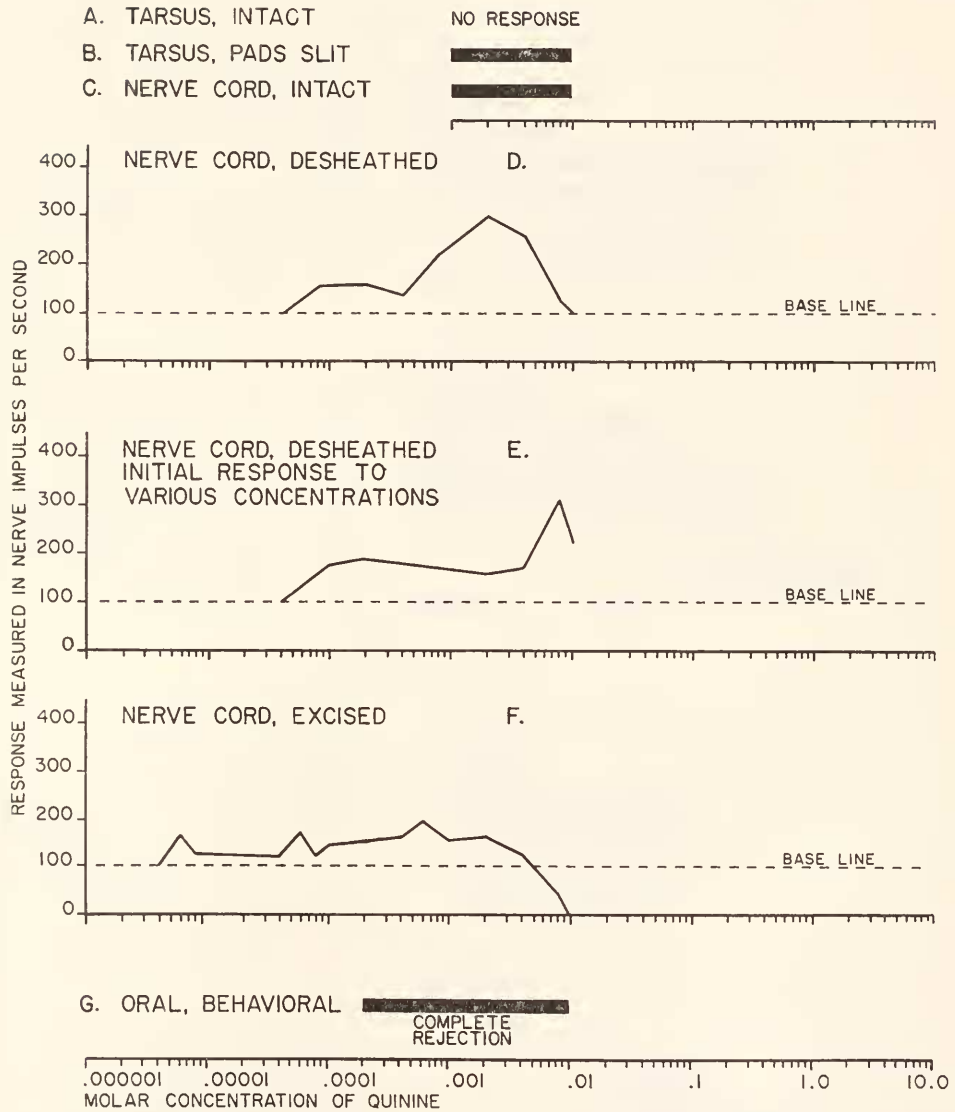

FIGURE 4. Responses of all types of preparations to quinine.

ical thresholds or whether they are due to osmotic or other physical changes. If they are due simply to increased osmotic pressure, then, conversely, threshold concentrations of all four substances should produce the same osmotic pressure. However, the computed osmotic pressures at threshold concentrations for sodium chloride, hydrochloric acid, sucrose and quinine are given in Table I and show a wide range of values for the four substances. This would seem to show conclusively that osmotic pressure was not the principal cause of the responses. The wide range

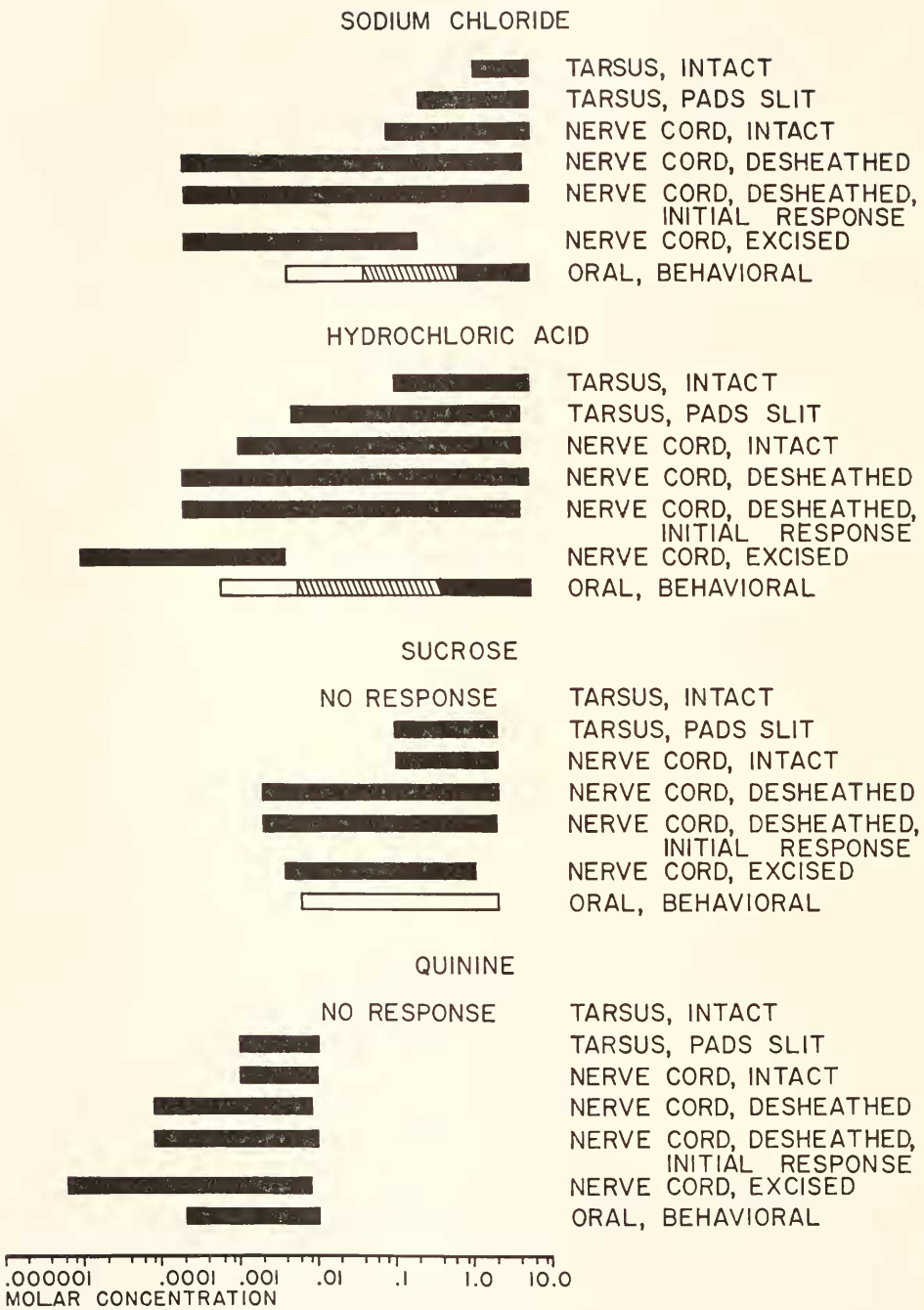

FIGURE 5. Responses of all types of preparations to sodium chloride, hydrochloric acid, sucrose and quinine

over which the thresholds extend seems to be further evidence against a single physical factor being responsible.

Secondly, some may question the physiological validity of certain of these thresholds. In particular the response of the nerves to sucrose may seem to be in direct conflict with the widespread practice among neurophysiologists of using "inert" sucrose to maintain the osmotic pressure of physiological saline solution in the absence of certain salts. However, it should be emphasized that at threshold concentrations the effects of these solutions are transitory. It is customary to allow a short period of time for any nerve preparation to recover from injuries of surgery, small osmotic changes produced when saline solution is substituted for blood, etc., before the activity is studied. Thus these transitory threshold responses might easily be overlooked or masked by other adaptive changes in activity. However, they show up clearly when separated experimentally from other possible causes of nerve activity. Moreover, when used in adjusting osmotic pressure the sucrose is substituted for some constituent of the solution, not added to the solution as was done in these experiments.

It is also possible that injury from operative techniques may have made the nerves more sensitive to chemical action. This seems unlikely in the tarsal preparations where injury was minimal and in the normal nerve cord where test substances were injected into an otherwise intact animal. Also, the Twarog window preparation was particularly designed to do a minimal amount of damage, and this is supported by the fact that thresholds for nerve cord exposed in the window with the sheath intact were no lower than those obtained by injection of the same substance. Desheathing may have torn some of the nerve fibers, but it seems very unlikely that these could have amounted to more than a small fraction of the total which responded to threshold concentrations. However, in the excised nerve cord a large number of connecting fibers were cut to remove it and these cut ends may have been very sensitive. This may have been a factor in the very low thresholds obtained from this type of preparation in response to stimulation by hydrochloric acid and quinine. If so, this would be interesting in itself, but these two thresholds are not essential to the general thesis of this paper.

Another important consideration is whether or not the thresholds from the intact tarsi and from exposed nerves are truly comparable to oral taste. The strongest argument in favor of this is the close correlation between the behavioral threshold and the thresholds of the various nerve preparations in response to a given chemical. This is most clearly shown in the responses to sucrose and quinine (see Figure 5) where the thresholds of the various nerve preparations for quinine are much closer to the behavioral threshold for quinine than to the behavioral threshold for sucrose and vice versa. The same may be said for responses to any other pair of test chemicals though it is more conspicuous in some pairs than in others.

Further correlation is found between the behavioral rejection thresholds for sodium chloride and hydrochloric acid and the activity produced in the nerve preparations by the same concentrations of the salt or acid. These concentrations suffice to raise the activity in desheathed nerves to a very high level and initiate activity in those nerves protected by a sheath or even by a sheath plus cuticle. This correlation seems to lend additional support to the relation between behavioral responses and the responses from nerve preparations. Further, it suggests that

when the stimulating chemical reaches a concentration which produces violent activity in the special taste receptor and begins to produce activity in all other nerves in the area, behavioral rejection sets in.

Results from the experiments with quinine do not show this correlation as clearly, while with sucrose we find violent activity in the nerve cord correlated with behavioral acceptance. However, this need not detract from the significance of the correlation between behavioral rejection and nerve response with salts and acids, since it is probable that the bitter and sweet molecules act on the receptors through quite different mechanisms than the salt and acid ions.

The protection afforded by the sheath in these cases brings us to a general summary of the factors which determine thresholds in different types of preparation. In the leg nerve preparation the tarsal cuticle was ruptured with the result that a lower concentration of chemical was needed to initiate nerve action. However, the sheath remained as a second line of protection. It was not practical to remove the sheath from the leg nerve, but a switch to the nerve cord showed similar thresholds. When the nerve cord sheath was removed, the concentration required for stimulation again dropped markedly. Viewing these experiments in general terms, it now seems probable that the *true* nerve threshold is the concentration of test substance which, when acting directly on the unprotected nerve, will produce a response. Any higher thresholds for protected nerves are measures of the concentration which must be applied outside the sheath or cuticle to produce the *true* threshold concentration at the nerve. Extrapolating to the normal oral taste receptors which control the behavioral thresholds, we may infer from the position of these behavioral thresholds, intermediate between those of sheath-protected and of desheathed nerves, that the nerves which govern them are not enclosed by normal sheath or cuticle but have more protection than bare nerve. This barrier may be to protect them from damage by high concentrations of chemicals, or it may be concerned with selection or differentiation between different types of taste stimulation.

In examining the curves lettered D and F, which represent the responses of desheathed nerve to the four chemicals tested, the most striking characteristic is the sharp drop in activity first seen after the threshold response. This rise to a peak of activity followed by a period of depression is repeated one or more times in varying degrees in all the curves before blocking occurs. A possible explanation is that the three or more peaks, particularly clear in the excised nerve cord preparations, may come from different groups of fibers each less sensitive or less exposed than the preceding. Each of these groups in turn could become active, reach a maximum, and then adapt or partially block to account for the peaks and depressions as the concentration of stimulating chemical is increased. In other words, parts of the nerve cord may be protected from chemical action by barriers comparable to the connective tissue sheath and the cuticle.

It is interesting to compare the behavioral thresholds with those obtained by Frings (1946) who also worked with cockroaches, using a different test method. In testing whether individual roaches would accept or reject test solutions offered them, Frings first determined the sucrose threshold by offering increasing concentrations of sucrose in water until an acceptable concentration was reached. This acceptance threshold was clear, but since individual roaches often refuse pure water, he had to use a different method to determine rejection thresholds. For this he-

TABLE II

Behavioral thresholds determined by two methods

Thresholds	Sucrose	Sodium chloride			Hydrochloric acid		
	Acceptance	Acceptance	Rejection		Acceptance	Rejection	
			Partial	Complete		Partial	Complete
Reported here	.006	.004	.04	.6	.0006	.006	.4
Frings	.007			.2			.02

selected a sucrose concentration far enough above threshold to be always acceptable (0.1 *M*) and offered this with the addition of increasing concentrations of sodium chloride until it was rejected. This he took as the rejection threshold for sodium chloride. He repeated the experiments with hydrochloric acid and a large number of other salts and acids, and Table II shows a comparison of his results with the behavioral thresholds determined in these experiments.

It is at once apparent that there is very good agreement on the determination of sucrose against water, but the present method gives much more information about the sodium chloride and hydrochloric acid. As might be expected, Frings' lowest records for rejection lie within the range reported here as partial rejection. Frings was aware of the existence of the lower acceptance thresholds reported here but was unable to determine them because he could not get consistent feeding responses without the use of sucrose. It was the use of a large population of roaches instead of individuals which made it possible to get consistent feeding responses in these experiments without the use of sucrose.

It is quite possible that these thresholds as well as those of the nerve preparations may vary with different diets. In these experiments the same diet was supplied throughout, but it seems probable that any dietary change would affect both behavioral and nerve thresholds equally so that the same relationships would remain.

The methods used do not show whether the impulses from the nerve cord arise in the axons, dendrites or somata of the activated nerve cells. Since all impulses above the amplifier noise level were counted, it is not possible from the present data to estimate either the size of the responding elements or their relative number in the total population of active neurons. From other studies (Roeder, 1948) it seems probable that many compounds exert their effects on the dendritic or somatic regions of the central neurons.

These studies seem to indicate that the chemical thresholds for nerves unspecialized for gustatory reception are as low as, or lower than, those of the specialized receptors. On this basis we may conclude that taste or contact chemoreception depends on two factors. One of these, sensitivity, is also held by the neurons unspecialized for gustatory perception. Therefore, we can study it in nerve tissue other than the complex receptors which offer considerable technical difficulty. Furthermore, we can bring to bear on this study the vast amount of work which has previously been done on nerve tissue. The other quality, discrimination be-

tween different substances, remains a property of the receptor mechanism. However, it is hoped that its study has been simplified slightly by separating from it the factor of sensitivity which has often been considered an integral part of this mechanism.

Summary

1. Application of increasing concentrations of sodium chloride to the normal intact tarsi of the American cockroach resulted in increased activity in the afferent fibers of the leg nerve when the threshold concentration was reached.

2. The threshold for this response was lowered by slitting the tarsal pads.

3. Although it may be presumed that there are no taste receptors on the nerve cord, when increasing concentrations of sodium chloride in saline solution were applied to an exposed section of intact nerve cord, it responded to a lower concentration than did the tarsal preparations.

4. The threshold of the nerve cord was further lowered by removing the connective tissue sheath which normally encloses it.

5. A section of the nerve cord, completely removed from the roach and exposed to the same concentrations of sodium chloride, responded at the same threshold concentration as the exposed nerve cord *in situ*, showing conclusively that the response did come from the nerve cord itself, not from adjacent chemoreceptors.

6. Behavioral experiments showed a response to the taste of sodium chloride at a threshold *higher* than that of the nerve cord preparations.

7. There was also an increase in the nerve activity from leg and nerve cord preparations in the same range of concentrations of sodium chloride which produced behavioral rejection.

8. Similar experiments with hydrochloric acid, sucrose and quinine, representing the sour, sweet and bitter sensations, also showed behavioral thresholds higher than those from the nerve cord preparations, and hydrochloric acid showed a correlation between nerve activity and behavioral rejection similar to that of sodium chloride.

9. It was concluded that high sensitivity to the four types of substances which produce the four taste sensations is inherent in nerves not normally connected with taste rather than being a special feature of the taste receptor, and that the basis for behavioral rejection may also be found in nerves not normally concerned with taste.

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